

# (12) UK Patent Application (19) GB (11) 2 328 023 (13) A

(43) Date of A Publication 10.02.1999

(21) Application No 9716749.8

(22) Date of Filing 08.08.1997

(71) Applicant(s)

The Victoria University of Manchester  
(Incorporated in the United Kingdom)  
Oxford Road, MANCHESTER, M13 9PL,  
United Kingdom

(72) Inventor(s)

Pankaj Madganiai Vadgama  
Keith Stewart Robert Warriner

(74) Agent and/or Address for Service

Peter Barry Tunnicliffe  
Chester Court, Church Close, BROADWAY,  
Worcestershire, WR12 7AH, United Kingdom

(51) INT CL<sup>6</sup>

C12Q 1/00 , G01N 33/487

(52) UK CL (Edition Q )

G1N NBPE N19B2Q N25B N25B3X N25DX N25D10  
U1S S1296 S1525

(56) Documents Cited

Derwent Abstract Accession Number  
86-085801/198613 and JP 61 33645 Sensors and  
Actuators B-Chemical, 1997, Vol44, No1-3 pages  
441-445. Medical & Biological Engineering &  
Computing 1995, Vol33, No.6, pages 811-821.

(58) Field of Search

UK CL (Edition P ) G1N NBMA NBPE NBPX  
INT CL<sup>6</sup> C12Q 1/00 , G01N 33/487 33/49 33/493  
ONLINE: WPI, BIOSIS, SCISEARCH

(54) Abstract Title

pH sensing devices

(57) A sensor device responsive to pH changes in the medium in contact with it is coated with diamond-like carb n. The device is particularly suitable for systems in which the pH change measured is the result of enzyme action, particularly when using urease to form ammonia from urea. The preferred sensor element is a solid state device particularly a field effect transistor in which the enzyme is bound on the surface of a semiconductor in conjunction with a conducting polymer, preferably polypyrrole. The impedance of the s nsor can be determined when in contact with a buffered solution of the sample to be examined, and can be used for determining urea levels in blood.

GB 2 328 023 A

The claims were filed later than the filing date within the period prescribed by Rule 25(1) of the Patents Rules 1995

## SENSOR DEVICES AND ANALYTICAL METHODS USING THEM.

This invention relates to sensor devices, and more particularly to improved sensor devices useful in analytical methods involving the detection or measurement of pH changes, especially in enzyme-based biosensor systems, and to analytical methods using them.

It is well known to make a variety of sensor devices in which an electrode is employed to provide output signals by which the presence or absence of an analyte in a sample can be determined. For this, an analyte (or a species derived from it) which is electro-active generates a detectable signal at an electrode, and this signal can be used as the basis for detection or measurement of the presence and/or amount of the analyte in a sample.

Bio-sensors have been found to be very successful in use for such purposes, especially when the bio-component is an enzyme. An enzyme has the advantage that it can be more specific to the analyte sought and also, when the analyte itself is not sufficiently electro-active, can be used to interact with the analyte to generate another species which is electro-active and to which the electrode can respond to produce the desired output signals. The classic example of such a sensor is the glucose oxidase enzyme-electrode, in which an immobilised glucose oxidase enzyme catalyses the oxidation of glucose to form hydrogen peroxide, which is then detected and determined by amperometric measurement of the effect it produces (increase in electrical current) at a polarised electrode (typically platinum). Measurements can be made conveniently in the usual manner, polarising the platinum electrode at 650 mV against an Ag/AgCl reference.

However, amperometric sensors and their use suffer from the limitation that other electro-active species present in a sample under examination (additional to the analyte sought) can cause interference, and also that enzymes do not always produce electro-active products. Consequently some

alternativ to amperometric transduction is desirable.

One such alternative which has been examined is the use of devices which are sensitive to changes in pH of media in contact with them. Changes in pH can be induced by the catalytic action of an enzyme (conveniently, an enzyme immobilised on the device) -- change being caused by the products formed by the action of the enzyme on the substrate analyte. However, although they do have great potential value, they are not entirely satisfactory in practice because they can still be adversely affected by other components of a sample. In particular, they are very susceptible to anything which can influence the buffering capacity of the sample. A component with a high or variable buffering effect can attenuate any changes in the pH as a result of the enzymic generation or consumption of H<sup>+</sup> ions or make them unpredictable. This can counteract or conceal the pH changes which it is desired to measure.

Such buffering components may be present in the sample itself or may be deliberately added, for example as a stabiliser or a preservative for the sample. Thus, a persistent problem encountered with pH-sensitive sensors is derived from the presence of any buffering salts in the sample matrix. In samples of high buffering capacity, the pH change induced by the enzyme is not detected by the sensing surface, hence no change in signal is observed and a false interpretation of the sample's composition is drawn. Furthermore, samples that have a variable buffering capacity will result in a significant "drift" in the sensor signals, again leading to practical limitations in their use.

There is, therefore, a need for a system which is able to overcome this buffering effect sufficiently to allow the desired measurement of the pH changes at the sensor. We have now found that the buffering effect can be decreased by application of a coating of a carbonaceous material to the material of the pH-sensitive detector element. The material which we have found to be very effective is already well-

known in itself and described in the art as "diamond-like carbon." It is conveniently referred to in the art by the abbreviation "DLC," and so is referred to in this manner through this specification.

5 Thus according to our invention we provide an improved sensor device which comprises a sensor which is responsive to changes in pH of media in contact with it, characterised in that the said sensor has a coating comprising a carbonaceous material having structural characteristics  
10 comparable with that of diamond.

Especially, the sensor which is responsive to changes in pH of media in contact with it is associated with an adjacent enzyme, so that interaction of the enzyme with the selected analyte can produce a change in pH at the sensor  
15 surface.

According to our invention we also provide an improved method for analysis of samples wherein a sample containing a selected analyte is brought into contact with a sensor which is responsive to changes in pH of media in contact with it  
20 and the change in pH induced by the interaction of the enzyme and the analyte is measured and used to provide a measure of the amount of the analyte present in the sample, characterised in that the said sensor has a coating comprising a carbonaceous material having structural  
25 characteristics comparable with that of diamond.

Especially, we prefer to use a form of carbonaceous material known as "diamond-like carbon" (DLC).

DLC is a form of amorphous carbon or a hydrocarbon polymer with properties approaching those of diamond rather  
30 than those of other hydrocarbon polymers. Various names have been used for it, for example "diamond-like hydrocarbon" (DLHC) and "diamond-like carbon" (DLC), but the term "DLC" appears to be the most common. It possesses properties attributable to a tetrahedral molecular structure  
35 of the carbon atoms in it, similar to that of diamond but with some hydrogen atoms attached. It has been described in

the art as being a designation for "dense amorphous hydrocarbon polymers with properties that differ markedly from those of other hydrocarbon polymers, but which in many respects resemble diamond" [J.C. Angus, EMRS Symposia Proc., 5 17, 179 (1987)].

The formation and application of the diamond-like carbon (DLC) to the membrane material as coatings or films for the purposes of the present invention may be carried out by methods known in the art. It is usually formed by 10 decomposition of carbon-containing compounds in gaseous or vaporised form (particularly hydrocarbon gases) induced by radiation or electrical fields.

Thus, it may be prepared from hydrocarbon precursor gases (e.g. propane, butane or acetylene) by glow-discharge 15 deposition, by laser-induced chemical vapour decomposition, by a dual-ion beam technique, or by introduction of the hydrocarbon gases directly into a saddle-field source. A saddle-field source is a source of ions produced by a collision between gas atoms excited by thermionic emission, 20 and this method is preferred because it allows heat-sensitive materials to be coated by a beam that is uncharged -- so facilitating the coating of insulating or non-conductive materials.

Its properties can vary according to the particular raw 25 materials used and its mode of formation. It can also be made in other ways, for example by sputtering solid carbon, as an alternative to dissociating hydrocarbon gases.

Further description of DLC (including its constitution, nature and properties, and the variations in its form which 30 can be made) and modes for its preparation, are to be found for example in the following published references (among others):-

(a) "Diamond-Like Carbon Applied to Bio-Engineering Materials;" A.C. Evans, J. Franks and P.J. Revell, of 35 Ion Tech Ltd., 2 Park Street, Teddington, TW11 0LT, United Kingdom; Medical Device Technology, May 1991,

pag s 26 to 29.

- (b) "Preparation and Properties of Diamondlike Carbon Films;" J. Franks; J.Vac.Sci.Technol. Vol.A, No.3, May/June 1989, pages 2307-2310;
- 5 (c) "Biocompatibility of Diamond-like Carbon Coating;" L.A. Thomson, F.C. Law, J. Franks and N. Rushton; Biomaterials, Vol.12, January 1991 (pages 37-40);
- (d) "Categorization of Dense Hydrocarbon Films;" J.C. Angus; E.M.R.S. Symposium Proc., 1987, Vol. 17, page 179;
- 10 Amorphous Hydrogenated Carbon Films, XVII, June 2-5 1987, Edited by P.Koide & P. Oelhafen.
- (e) "Properties of Ion Beam Produced Diamondlike Carbon Films;" M.J. Mirtech; E.M.R.S. Symposium Proc., 1987, Vol. 17, page 377;
- 15 (f) "Diamond-like Carbon - Properties and Applications;" J. Franks, K. Enke and A. Richardt; Metals & Materials (the Journal of the Institute of Metals); and
- (g) U.S. Patent No. 4490229; M.J.Mirtich, J.S.Sorey & B.A.Banks.
- 20 The convenient source of the DLC carbon is a hydrocarbon gas or vapour, especially one which is readily decomposed by an electric field or discharge. A very convenient source gas is acetylene, though others may be used if desired. Individual hydrocarbons or mixtures thereof may be used, and diluent gases may be added if desired, and the decomposition/deposition procedure may be carried out at pressures at atmospheric or above or below atmospheric, as may be found most suitable for particular instances and the sensitivity of the enzyme used.
- 25 30 The sensor which is responsive to changes in the pH of media in contact with it may be any of those known in the art. An example of this type of sensor is that known as an enzyme field effect transistor ("ENFET"). Such devices are well known. Here, an enzyme is immobilised on the surface of a semiconductor material (which is typically made of silicon, though other materials may be used) carrying inter-

digitating electrodes with a bridging conducting polymer film. The change in pH resulting from enzymic activity alters the conductivity of this material -- which is detected by monitoring the extent of electric current flow 5 through it (i.e. the "drain current").

ENFET devices have advantages over devices used for amperometric detection -- notably a lower susceptibility to electro-active interference and also usefulness with enzymes which do not form electro-active products. They have so far 10 been based primarily on silicon semiconductor technology, but alternatives to silicon may be used if desired, for example organic conducting polymer films.

Some conducting polymers have properties similar to semiconductors, in that it is possible to "switch" them 15 repeatedly between conducting and insulating states. This switch can be induced by change in the polymer redox state and/or their degree of protonation (pH). It is this latter property which has been the basis for the polymers used in ENFET type devices.

20 An example of an especially useful conducting polymer is poly(pyrrole). This is made in known manner, for example by electro-polymerisation or oxidation of pyrrole, usually in aqueous solution - the polymer precipitating as it is formed. Other known conducting polymers can be used if 25 desired.

Another example of its use is in a sensor sensitive to penicillin, based on poly(pyrrole) with an overlaying layer of immobilised penicillinase. Upon exposure to penicillin, the polymer becomes more conductive (through protonation) 30 which is detected by an increase in drain current passing through the conducting polymer film. As an alternative to measuring drain currents, impedance (resistance) measurements can be used as the measure of polymer conductivity as, in principle, both drain current and 35 impedance provide the same information regarding the film conductivity, though the latter is recognised as being a

more sensitive technique.

The thickness of the DLC coating may vary according to the particular requirements desired for the performance of the sensor and the system to be analysed. It should be 5 permeable enough to let the relevant components pass through but not so thick as to prevent adequate permeation; the optimum can be found for any particular case by simple trial. Thus, the thickness of the DLC coating or deposit may be in the range 0.01 to 1.0 nm, but thicker or thinner 10 coatings may be used if desired. A typical and convenient coating deposit is one approximately 0.5 nm thick, but this is not necessarily the optimum for all purposes. The optimum thickness in any particular case will depend upon such factors as the nature (physical and chemical) of the 15 material upon which the DLC is deposited, and its porosity or permeability, and the particular enzyme and substrate concerned, and the characteristics appropriate to the intended use of the sensor.

The coating with DLC is conveniently carried out at a 20 rate which allows the deposit to adhere to the membrane material and form a coating of the desired thickness - preferably also evenly coated so as to cover substantially all the surface without leaving any areas too thinly covered or even un-covered.

25 When using acetylene as a source, for example, the deposition may be carried out at a rate of up to 0.5 nm per hour, though higher or lower rates may be used if desired.

The invention is applicable to a variety of enzyme systems which act to form acidic or basic products which can 30 change the pH of the media in which they act. The principal example is an enzyme which hydrolyses a substrate (especially urea) to form ammonia, as ammonia is strongly basic, but can also be applied to systems in which other pH-altering compounds can be formed, for example amines. The 35 commonest enzyme we find useful is urease, which hydrolyses urea to carbon dioxide and ammonia.

The improved sensors according to the present invention have the advantage that their range of linear response (i.e. the relationship between the sensor output signal and the amount of the substrate analyte) is extended, in comparison 5 with known sensors. This makes them much more useful in practical clinical or laboratory conditions; by their use, the ease, speed and reliability of measurement or detection can be extended considerably.

The benefits are described herein with particular 10 reference to the urea/urease system, which is a particularly useful example for its use, but this should not taken to mean that the invention is limited to this system.

In a urea/urease system, using the improved sensor of the present invention, typically this range of linear 15 response is increased from 2 mM to 6 mM of urea.

In practice, it is usual for some samples to be diluted down in order to make them fall within the working range of the sensing device. Thus, in some clinical applications (e.g. blood samples) the levels of urea analyte present may 20 be in a region (e.g. 20 mM urea) which too high for sensors to be used satisfactorily. Though the present invention does not eliminate entirely the need for some dilution of a sample, nevertheless it does reduce it considerably because the DLC-coated sensors of our invention have an increased 25 range of linear response. The result of this is that sample dilution becomes far less critical and one can simplify considerably the previous need to carry out multiple tests (by several dilution steps) to determine the optimum degree of dilution for the particular sample under test.

An increase in the ease and speed of urea detection and measurement at high concentrations can be of value for patients with acute or chronic renal failure, where knowledge of blood urea levels provides insight into the extent of renal damage. It is also of value in monitoring 35 urea during dialysis therapy.

In use, the electrode of our invention can be used to

carry out the method of our invention by simple immersion in a predetermined volume of a buffer solution to which the sample to be analysed has been added, and applying an alternating voltage (AC), with a superimposed bias voltage,  
5 so that measurements of the impedance can be made.

This measurement procedure may be done in conventional manner, using conventional equipment. Measurements of th impedance (resistance) may be taken and the measurements taken and recorded as desired, intermittently or  
10 continuously. For this, conventional apparatus may be used. Samples of the media for examination may be obtained by standard methods. The quantity of sample should be sufficient to cover the sensor and the current measured at a fixed time or after a stable response has been achieved.  
15 Likewise, samples of other media may be obtained in any convenient manner and brought into contact with the sensor of the present invention for the purpose of component detection.

If desired, the procedure may also be calibrated by use  
20 of solutions containing known amounts of the substances sought, and its accuracy this checked and confirmed. Likewise, the procedure may be carried out using known amounts of compounds which are considered to be potentially troublesome by their expected ability to interfere with the  
25 measurement, so that the degree of interference (if any) can be established. Conventional apparatus may be used, for the cell, electrodes and the measurement and recording of the impedance relationships for the samples under test. Measurements may be made continuously or intermittently, as  
30 desired.

The most suitable bias voltage is +0.4V against an Ag/AgCl reference.

The pH of the sample/buffer mixture being examined may vary in the pH rang 5 to 8 within which lies the pH optimum  
35 of many enzymes. Howev r, the s lected pH for an assay mixtur may be chosen to be close to 7.4, which is the

physiological pH of blood plasma.

The sample under examination may be stirred or not, as desired or convenient.

The measurement procedure for use of the sensors of our invention may be carried out over a considerable range of temperatures, for example in the range 20 to 40 degrees C.. The medium comprising the sample examined is commonly aqueous, but need not necessarily be so, and an organic solvent may be used if desired (as such, or in admixture with each other and/or water) provided it is an electrolyte and dissolves any desired reagents, but is not medically relevant to the assay carried out.

Diamond-like carbon coatings have the advantages of a high degree of inertness and also a high degree of bio-compatibility.

THE DLC coating also serves as a mechanical barrier against abrasion of the enzyme layer; an ultra-thin, low permeability layer that controls the permeation of the analyte without adversely extending response times; reduces access to the electrode by buffer interferents; reduces protein and cell bio-fouling of the electrode and so increases bio-compatibility.

The invention is illustrated but not limited by the following Examples.

25

**EXAMPLE 1.**

**A Urea-Sensitive Urease/Poly(pyrrole) Impedimetric Sensor.**

30 **Initial Starting Device.**

An inter-digitating electrode ("IDE") is used as the starting device. This comprises a base of an insulating material carrying on its surface two electrically separate conducting arrays which intermesh with each other so that they form, over the surface of the base, a pattern combining the two conducting elements which do not make contact with

each other. This pattern area forms a "sensing area" which can respond to the conductivity of any media spread across it and contact both conducting element arrays. The two conducting elements are connected electrically to simple 5 terminals by which they can be connected into an electrical circuit, for example for measurement of the electrical impedance (resistance) between the two element arrays. It was made as follows:-

Gold inter-digitated electrodes were fabricated by 10 photo-lithography and consisted of a gold layer (500 nm thick) deposited on to an insulating silicon substrate which comprised a 1 nm thermal oxide layer on which a 0.16 nm layer of silicon nitride had been deposited. A 30 nm chromium layer assured adhesion of the gold on to this 15 underlying substrate. The IDA consisted of 50 digits, each 10  $\mu\text{m}$  wide and 500  $\mu\text{m}$  long, separated by a 15  $\mu\text{m}$  gap.

Sensor construction. (Forming poly(pyrrole)/urease films).

Poly(pyrrole) films were made on the surface of the IDE 20 by deposition from a de-oxygenated aqueous solution containing pyrrole (100 mM) sodium dodecyl sulphate (2 mM) and urease (1350 U). The pH of this solution was adjusted to 7, using 0.1 M sodium hydroxide, and polymerisation was achieved by potential cycling between -0.25 V and +0.9 V 25 against an Ag/AgCl reference at a scan rate of 50 mV/s.

During the electro-polymerisation of pyrrole the formed poly(pyrrole) precipitates from the aqueous solution on to the electrode surface and eventually forms a smooth film bridging the inter-digitating electrodes. With this polymer 30 deposition on to the electrode surface, the urease enzyme layer becomes entrapped and thereby immobilised.

It was found that the growth of conducting polymer across the electrode gap could be more readily achieved by using a three-electrode system with the IDE as the working 35 electrode, a separate counter (platinum flag, 2 x 3 cm) and an Ag/AgCl reference. The coated electrode was washed with

a saline buffer (50 mM, at pH 7.4, containing 50 mM potassium chloride) to avoid enzyme de-naturation and to remove excess reagents.

In forming these conducting polymer films the monomer 5 is oxidised, which makes it reactive and able to combine with further oxidisable monomers. Eventually the polymer becomes insoluble and is deposited on to the surface of the electrode and its support. In the case of an array of inter-digitated electrodes, the polymer deposit grows across 10 the digits and thereby provides an electrical connection between them. Thus, current flows from adjacent digits and through the polymer, and hence any change in the polymer conductivity will be recorded by a change in resistance. In the present case, it is the ammonia formed 15 by the action of the enzyme on urea that de-protonates the poly(pyrrole) polymer -- thereby resulting in an increase in its resistance to current flow between adjacent electrodes which is detected by impedance measurement.

The resulting sensor device was coated with a layer of 20 diamond-like carbon 0.5 nm thick by deposition of the DLC by striking a plasma in an atmosphere containing hydrocarbon vapour (methane). The DLC was deposited at a rate of 1 Angstrom a second, and the resulting DLC was in an insulating, inert, flexible and bio-compatible thin film.

25

#### Principle of Operation.

Urease catalyses the hydrolysis of urea to form ammonia and carbon dioxide. The ammonia thus produced de-protonates the supporting poly(pyrrole) film and, as a result, produces 30 a decrease in the polymer conductivity (an increase in its resistance), which can be detected by impedance measurement. On contact with a sample solution containing urea, the solution permeates through the polymer and the urea in it interacts with the urease, and the ammonia so formed then 35 alters the impedance as described above.

Procedure.

The IDE coated with urease/poly(pyrrole) prepared as described above is connected electrically, by its terminal connections, to a conventional apparatus for measuring the 5 impedance (resistance) of the bridging conducting polymer film over the inter-meshed array of conducting elements, and then dipped into an aqueous solution containing urea as analyte, and the impedance (resistance) of the coated IDE is then measured. the impedance measurements provide a measure 10 of the urea content. The range of urea concentrations used for calibration was 1 to 20 mM urea; for test purposes, a concentration of 5 mM urea was convenient for use.

It was found that the "urea response" (the amount of urea deduced from the measurements) of a sensor without any 15 DLC coating is high for a sample solution of a low buffering capacity but this decreases progressively with increasing buffering salt concentration. In contrast to this, sensors with a DLC coating layer the responses are lower but, more importantly, are almost independent of solution buffering 20 capacity (content of buffer salts). This was well illustrated using a series of sensors having a DLC coating 0.5 nm thick.

----- 0 -----

## WHAT WE CLAIM IS:-

1. A sensor device which comprises a sensor which is responsive to changes in pH of media in contact with it, characterised in that the said sensor has a coating comprising a carbonaceous material having structural characteristics comparable with that of diamond, particularly known as diamond-like carbon.  
5
2. A sensor device as claimed in Claim 1 wherein the sensor which is responsive to changes in pH of media in contact with it is associated with an adjacent enzyme, so that interaction of the enzyme with the selected analyte can produce a change in pH at the sensor surface.  
10
3. A sensor device as claimed in Claim 2 wherein the sensor which is responsive to changes in the pH of media in contact with it is an enzyme field effect transistor ("ENFET").  
15
4. A sensor device as claimed in Claim 3 wherein the enzyme field effect transistor contains poly-pyrrole as its conducting polymer,
- 20 5. A sensor device as claimed in any of Claims 1 to 4 wherein the thickness of the diamond-like carbon coating is in the range 0.01 to 1.0 nm, typically approximately 0.5 nm.
6. A sensor device as claimed in any of Claims 2 to 5 wherein the enzyme is one which hydrolyses a substrate (especially urea) to form ammonia or an amine - especially urease, which hydrolyses urea to form ammonia - which is then detected by its basic properties.  
25
7. A sensor device which comprises a sensor which is responsive to changes in pH of media in contact with it and has a coating comprising a carbonaceous material having structural characteristics comparable with that of diamond substantially as described with reference to the Examples.  
30

8. Method for analysis of samples wherein a sample containing a selected analyte is brought into contact with a sensor device as claimed in any of Claims 1 to 7.
9. Method as claimed in Claim 8 wherein the sensor device  
5 is contacted with the sample to be examined by simple immersion in a predetermined volume of a buffer solution to which the sample to be analysed has been added, and applying an alternating voltage, with a superimposed bias voltage, so that impedance measurements can be made.
10. Method as claimed in Claim 8 or Claim 9 wherein the species to be detected and determined is urea, and the sensor device contains the enzyme urease.
11. Method as claimed in Claim 10 wherein the procedure is applied to the determination of urea levels in blood.
15. 12. Method for the analysis of samples using a sensor device which comprises a sensor which is responsive to changes in pH of media in contact with it and has a coating comprising a carbonaceous material having structural characteristics comparable with that of diamond, substantially as described with reference to the Examples.



The  
Patent  
Office  
16

Application No: GB 9716749.8  
Claims searched: 1-12

Examiner: Dave Mobbs  
Date of search: 8 July 1998

**Patents Act 1977**  
**Search Report under Section 17**

**Databases searched:**

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK CI (Ed.P): G1N NBMA, NBPE, NBPX.

Int CI (Ed.6): C12Q 1/00; G01N 33/487, 33/49, 33/493.

Other: ONLINE: WPI, BIOSIS, SCISEARCH.

**Documents considered to be relevant:**

Category	Identity of document and relevant passage	Relevant to claims
X	Derwent Abstract Accession Number 86-085801/198613 and JP 61033645 (Sumitomo Chem Ind. KK)	1 at least
X	Sensors And Actuators B-Chemical, 1997, V44, N1-3, pages 441-445, University of Hannover, Institut Halbleitertechnol & Werkstoffe Elektrotech. Diamond-like carbon gate pH ISFET.	1 at least
X	Medical & Biological Engineering & Computing, 1995, V33, N6, pages 811-821, Keele University et al., Hemocompatibility of Invasive Sensors.	1 at least.

- X Document indicating lack of novelty or inventive step  
Y Document indicating lack of inventive step if combined with one or more other documents of same category.  
& Member of the same patent family

- A Document indicating technological background and/or state of the art.  
P Document published on or after the declared priority date but before the filing date of this invention.  
E Patent document published on or after, but with priority date earlier than, the filing date of this application.

**THIS PAGE BLANK (USPTO)**